

研究论文

Research Article

Shotgun 蛋白质组学技术在致病性烟草青枯菌分泌蛋白研究中的应用

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摘要 烟草青枯病是由茄科劳尔氏菌(*Ralstonia solanacearum*)引起的一种毁灭性的烟草种植灾害, 可造成烟草的巨大减产。本实验首次采用 shotgun 蛋白质组学技术对致病性烟草青枯菌菌株的分泌蛋白进行系统分析, 结果鉴定得到了 384 个蛋白质。按照青枯菌的致病特点和侵染过程将这 384 个蛋白分为糖蛋白、信号肽蛋白和跨膜蛋白等 7 个功能类群。其中, 17 个被前人研究报道为与青枯菌致病相关的蛋白质, 表明使用 shotgun 蛋白质组学技术可有效的分析青枯菌分泌蛋白, 具有可行性和有效性。327 个迄今为止尚无文献报道其与青枯菌致病性相关, 40 个为未知蛋白。这些研究结果为进一步揭示青枯菌对烟草的致病机理及抗病基因的功能克隆提供了依据。

关键词 烟草青枯菌; 分泌蛋白; 致病性; Shotgun 蛋白质组学

The Application of Shotgun Proteomics in Tobacco *Ralstonia Solanacearum* Secretory Proteins

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Abstract *Ralstonia solanacearum* (synonym pseudomonas solanacearum) is a widely spread soil-borne plant pathogen which causes “bacterial wilt”, a major bacterial plant disease that greatly impact the production of tobacco. In this study, we analyzed the structure and function of *Ralstonia solanacearum* secreted proteins by employing shotgun technology, and identified 384 proteins. Base on the pathogenic characteristics and infection process, the 384 proteins were classified into seven clusters: such as glycoprotein, signal peptide protein, transmembrane protein. 17 of these proteins were reported in the previous studies as *Ralstonia solanacearum* pathogenicity proteins, 327 have been identified for the first time as pathogenicity proteins of *Ralstonia solanacearum*, and 40 were uncharacterized proteins. This result provides us with the basic information for further studying the pathogenic mechanism and for conducting functional cloning of the resistant-related genes in tobacco to *Ralstonia solanacearum*.

Keywords Tobacco *Ralstonia*; *Solanacearum*; Secretory proteins; Pathogenicity; Shotgun proteomics

烟草青枯菌(*Ralstonia solanacearum*)是在世界范围内危害烟草生产, 引起烟草青枯病的主要致病菌, 致使每年烟草种植的大量减产。该病菌还会危害其它诸如土豆, 番茄等世界主要经济作物(李林章等, 2005)。因此, 明确青枯菌的致病机理, 阐明烟草的抗病基因功能, 对高产栽培调控和抗病分子

育种都有十分重要的意义。到目前为止, 国内外已经从基因定位和分子标记辅助育种(扬友才等, 2006; Jiang et al, 2007)、基因组序列比对(Remenant et al., 2010; Fujiwara et al., 2008; Guidot et al., 2007)、转录组学(Guidot et al., 2009)、蛋白质组学(宋浩等, 2010; Dahal et al., 2010)以及抗性基因转化(Zhang et al.,

2009; Huang et al., 2007)等多个方向对青枯病的致病机理开展了大量深入的研究。这些研究表明青枯菌之所以能够引起多种植物的致死性枯萎, 主要是通过根、茎和伤口或次生根的根冠进入植物体后, 分泌大量胞外蛋白和多糖感染寄主的木质部, 然后在整个维管束中蔓延, 引起严重的萎焉最终导致植物死亡(Denny et al., 1990; Poueymiro and Genin, 2009)。同时发现青枯菌分泌蛋白多为病原微生物与植物受体蛋白起作用的激发子和其它致病因子(Kang et al., 1994; 宋浩等, 2010), 所以分析这些分泌蛋白的组成和功能对于人们认识烟草青枯菌的致病机理和建立有效地防治手段有着重要的意义。目前青枯菌(GM1000菌株)全基因组测序的完成(Salanoubat et al., 2002), 使得从蛋白质组水平分析和研究该菌的致病机制成为可能。近年来, 以不同青枯菌菌株以及宿主为研究对象的比较蛋白质组学研究已取得了一些新的进展(Dahal et al., 2009; Kang et al., 2008; Monchy et al., 2006; 宋浩等, 2010), 但尚未见有关利用蛋白质组学方法研究青枯菌分泌蛋白的报道。随着蛋白质组学的日益发展, 利用现代质谱技术可以快速, 有效, 高通量地鉴定生物样本的蛋白质。而蛋白质组学中常用的质谱shotgun (鸟枪法)技术可以通过对复杂的蛋白质样品进行酶解形成肽段, 经过广泛的分级后, 由串联质谱进行鉴定(夏其昌和曾嵘, 科学出版社, pp.278), shotgun蛋白质组学技术已经成为研究复杂蛋白质混合物的有力工具(Wu and MacCoss, 2002)。本实验首次采用shotgun蛋白质组学技术对致病性青枯菌分泌蛋白进行系统研究, 为进一步阐述烟草青枯菌的致病机理提供了实验数据和理论基础。

1 结果与分析

1.1 菌体的培养

在进行青枯菌菌体扩培前, 参考陈晓敏等的方法首先使用 2,3,5-三苯基氯化四氮唑培养(TTC)琼脂平板培养基从表型上验证其致病性, 平行接种的青枯菌菌体于 TTC 琼脂平板上 30℃ 培养(陈晓敏等, 2000), 48 h 后培养结果如图 1 显示, 致病性菌株呈现典型的周围光亮白边中心红点特征, 结果与文献报道的一致(李文溶和段迺雄, 1988), 说明本实验

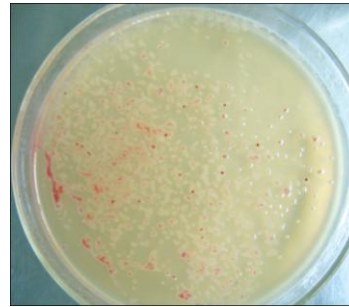


图 1 TTC 琼脂平板培养基上的致病青枯菌
 注: 培养时间: 48 h; 培养温度: 30℃

Figure 1 The pathogenic *Ralstonia solanacearum* on the TTC agar medium

Note: The cultivate time was 48 h; The cultivate temperature was 30℃

所用菌体样品符合要求。

1.2 分泌蛋白质提取以及 SDS-PAGE 结果

致病性青枯菌接种于分泌培养基中, 收集培养基并用 Amicon Ultra-15 (截留分子量 5 K)超滤装置浓缩超滤含有分泌蛋白的培养基后定量, 结果在 200 mL 的分泌培养基中, 致病青枯菌分泌了多达 50 μg 的蛋白质。致病性青枯菌培养基蛋白质 SDS-PAGE 电泳结果如图 2 所示: 致病性青枯菌分泌蛋白大部分集中在 27 KDa 与 97 KDa 之间, 在

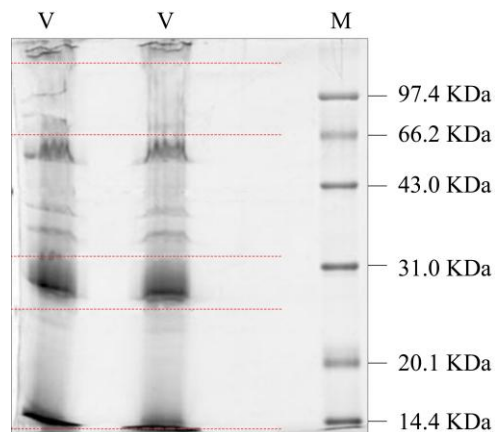


图2 致病性青枯菌分泌蛋白质SDS-PAGE结果

注: V: 致病青枯菌分泌蛋白; M: 蛋白质分子量Marker; 每个泳道分成5个条带

Figure 2 The SDS-PAGE result of the pathogenic *Ralstonia solanacearum* secretory proteins

Note: V represents the secretory proteins of pathogenic *Ralstonia solanacearum*; M represents protein molecular weight marker; Each gel lane was divided into 5 regions

27 KDa 左右有高丰度蛋白的存在。根据 SDS-PAGE 电泳蛋白条带的分布, 我们将其分为 5 个条带组进行后续的酶解。

1.3 LC-MS/MS 鉴定结果

致病青枯菌分泌蛋白质样品经 SDS-PAGE 分级后, 酶解产物使用 LC/MS/MS 鉴定, 鉴定控制假阳性率为 $P < 0.05\%$, 并且控制至少含有两个以上唯一肽段的蛋白为可信蛋白, 经过这些严格筛选, 最后得到 384 个蛋白质(其中 69537 个 MS/MS 色谱图, 得到 2314 个肽段, 鉴定到 485 个唯一肽段), 鉴定结果见附表 1。按照青枯菌分泌蛋白的输出和传递过程以及分泌蛋白的致病特点(Chun-Mei et al., 2002), 我们将这 384 个蛋白质分为以下 7 大类: 糖蛋白(3%)、信号蛋白(6%)、跨膜蛋白(6%)鞭毛蛋白(6%)、效应蛋白(2%)、毒性蛋白(4%)和其他蛋白(73%)。

1.3.1 糖蛋白

青枯菌在侵染宿主过程中首先分泌大量胞外多糖, 胞外多糖影响和阻碍植物体内的水分运输, 特别是易于对叶柄结和小叶处较小孔径的导管穿孔板造成堵塞, 因而引起植株萎蔫(Denny et al., 1990)。在我们的结果中, 发现有 3% 的蛋白(11 个)与糖代谢过程相关。其中胞外多糖分泌蛋白 P58597 已被证实为青枯菌致病性相关蛋白(Orgambide et al., 1991)。同时我们首次发现了另外 5 个胞外多糖分泌蛋白 A3RX63/Q8XT40/Q8XTU8/Q8XX75/Q8XQI5 (附表 1)。在青枯菌分泌的与糖代谢相关蛋白中, 除了胞外多糖分泌蛋白外, 还有一大部分为具有很强分解能力的糖蛋白, 它们主要参与对宿主植株表层细胞壁的分解作用, 这样使得病原菌可以成功侵入植株, 到达质外体, 通过向胞外空间甚至植物细胞内释放效应蛋白(effector proteins) (Dangl and Jones, 2001; Hammond-Kosack and Parker, 2003)。在我们的结果中也发现了 5 个类似的具有分解能力的糖蛋白: 溶果胶酶 P58601、多聚半乳糖醛酸酶 Q8XRJ8、PheB 多聚半乳糖醛酸酶 Q53241 和类枯草杆菌素蛋白酶 A3RTC4 和 B2UBR3。其中类枯草杆菌素蛋白酶 A3RTC4 和 B2UBR3 蛋白属于丝氨酸蛋白酶家族, 在稻瘟病菌, 烟曲霉等一些病原菌

致病过程中的作用已有报道, 具有很强的分解细胞壁功能(魏艺聪等, 2009)。

1.3.2 信号蛋白和跨膜蛋白

在青枯菌成功侵入宿主质外体以后, 通过精巧的蛋白传输系统, 产生各种与致病性相关的效应蛋白 (effector proteins) 和毒性蛋白(Koster et al., 2000)。这些效应蛋白和毒性蛋白在传递之前必须被青枯菌膜蛋白上的信号肽(signal peptide)识别以后才能进入正常的分泌途径。信号肽(signal peptide)是跨膜蛋白起始密码子后, 有一段编码疏水氨基酸序列的 RNA 片段, 该片段所编码的肽段。新合成的蛋白质通过该信号肽的识别进入到正常的分泌途径。所以信号肽和跨膜蛋白在病原和寄主之间进行信息传递发挥, 致病和诱导寄主的抗病反应中起着重要的作用(Huang et al., 2007)。在我们得到的 384 个青枯菌分泌蛋白中, 发现有 12% 的蛋白(47 个)属于信号蛋白或者跨膜蛋白。其中有 23 个信号肽蛋白(A7CK23/A7CLD6/A7CM55/B2UDH0/Q8XPI6/Q8XQJ7/Q8XQM1/Q8XR59/Q8XRC9/Q8XRS3/Q8XS79/Q8XUC7/Q8XV98/Q8XUU4/Q8XVH6/Q8XV V9/Q8XW22/Q8XX33/Q8XYK2/Q8Y0Z1/Q8Y0Z3/Q8Y1X9/Q8Y382)和 24 个跨膜蛋白以及跨膜相关蛋白(Q8XRS6/Q8XSE4/Q8XT54/Q8XTF1/Q8XTX0/Q8XTY6/Q8XWU0/Q8Y119/Q8Y175/Q8Y1N4/Q8Y2A7/Q8Y2I4/A7C9Y2/A7CG74/A7CF52/B2U9G4/B2UFJ2/B5RXC1/B5SER5/B5RXI8/B5S060/B5SKP 6/Q8XR13/Q8XR28) (附表 1)。

1.3.3 鞭毛蛋白

细菌的分泌蛋白经过膜蛋白上的信号肽识别后进入分泌系统。细菌的分泌系统是转移所分泌蛋白和其它分子最重要的系统, 主要有 4 种类型的分泌系统(Genin, 2002), 其中 II 型分泌系统(T2SS)和 III 型分泌系统(T3SS)对青枯菌的致病性贡献最大(Genin and Boucher, 2004)。细菌的分泌蛋白在通过分泌系统进行分泌过程中, 细菌的附属物鞭毛(flagellin)起到了不可或缺的媒介作用(Chun-Mei et al., 2002)。鞭毛蛋白(flagellin protein)作为病原细菌毒力因子鞭毛的组成成分, 参到分泌系统通路, 同时它的运动性也是形成细菌侵染宿主的主要因

素之一(Orans et al., 2010)。在我们的研究结果中,发现了 6%的青枯菌分泌蛋白(23 个)为鞭毛蛋白:鞭毛蛋白 B5SA46/Q8XST5、鞭毛蛋白片段 Q0QY4/2Q0Q2Y5/Q0Q2Y8/Q0Q2Z3/Q0Q2Z4/Q0Q2Z6/Q0Q2Z8/Q0Q318/Q0Q320/Q0Q3/Q0Q326/Q83UT8/Q847Y78、L-环鞭毛蛋白 B2UJW8/A7C9I6/A3RUG8、菌毛转运蛋白 B5RW01/B5SHR2/Q8XVZ3 和 4 型菌毛蛋白 B5SGQ4。

1.3.4 效应蛋白

通过传输系统的分泌和传递作用,细菌的效应蛋白(effector protein)便成功注入宿主细胞内。这些效应蛋白表现出多种毒性效应,包括促使宿主细胞骨架的改变,破坏信号转导途径和抑制凋亡,并在宿主细胞建立适于寄生的亚细胞环境等(Mota and Cornelis, 2005)。细菌正是通过这些效应蛋白的毒性作用来干扰植物正常的生理状态,抑制植物的防御反应,促进自身的大量增殖(曹鹏等, 2009)。在 384 个青枯菌分泌蛋白中,我们得到了 8 个效应蛋白,其中 Lrr-gala 家族 3 型分泌蛋白 B5S4F6(Mukaihara and Tamura, 2009)和 popf1 分泌蛋白 Q8XPT2 (Meyer et al., 2006)已经被证实是青枯菌致病性效应蛋白。同时我们也首次得到了另外 6 个效应蛋白: B7ZJG6/C0SPP3/Q8XRC8/Q8XZK9/C0SPN3/A3RZU5 (附表 1)。

1.3.5 毒性蛋白

除了以上提到的通过调节宿主细胞的代谢过程关键酶的活性来实现致病过程的效应蛋白外(Mota and Cornelis, 2005),我们在 384 个青枯菌分泌蛋白中发现了 15 个直接进行毒性作用的毒性蛋白。文献报道蛋白 PopA(Q84IE8 和 Q9RBS0)可以明显增强青枯菌的致病性,随后的实验发现在 *PopA* 基因中插入特殊片段后可以明显抑制疾病形成,致使宿主的青枯现象明显改善(Kanda et al., 2008)。蛋白 B5SAX2 (rela/spot domain protein)被发现在细菌侵入宿主和生长的过程中一直保持着生物活性(Sun et al., 2009)。外膜蛋白 OmpW (A7CFB0 和 B2UFP6)和外膜蛋白 OmpA/MotB (A7CM53 和 B2U879)被证实主要是与宿主的受体分子进行相互作用(Sun et al., 2009)。蛋白 PilA (A3RQ41)和 PilT (A3RTF1)已

经被很明确的提到属于青枯菌的毒性蛋白(Kang et al., 2002)。蛋白 FtsN (A3RWZ4)和异青霉素 N 差向异构酶(A3RQV4)有文献报道主要参与宿主的细胞分裂等过程,但是否与青枯菌的致病性相关还需进一步验证(Derouaux et al., 2008)。血溶素样蛋白 Q8XT20/Q8Y2T5/Q8Y377/Q8Y378 (hemolysin-type protein)据报道主要参与大肠杆菌的致病过程(Blum et al., 1995)。

2 讨论

烟草青枯病是由青枯菌(*Ralstonia solanacearum*)引起的一种毁灭性灾害,而青枯菌的胞外分泌蛋白多为致病过程中的关键因子(Kang et al., 1994; 宋浩等, 2010)。通过对分泌蛋白的检测与分析,可能对阐明分泌蛋白在青枯菌致病过程中的作用机制有较大帮助。目前与青枯菌分泌蛋白相关的蛋白质组学研究还没有报道,本研究是在这一领域进行的初步探索。

Shotgun 技术是近年来发展迅速的蛋白质组学方法(Gavin et al., 2002),较传统的 2D-MS (双向电泳-质谱)蛋白质组学策略具有高通量、易操作性、快速性以及很好的普适性等优点(Blonder et al., 2002; Janini et al., 2003)。通过 shotgun 蛋白质组学的应用,我们得到了 384 个具有高可信度的致病性烟草青枯菌菌株分泌蛋白,这为烟草青枯菌致病性研究积累了重要资料。但是,shotgun 蛋白质组学技术本身也有一些不足之处,比如蛋白质直接酶切得到的肽段混合物过于复杂,较难实现对细胞全部蛋白质的识别和鉴定等(Matallana-Surget et al., 2010),所以,在青枯菌分泌蛋白的提取、分离纯化及分析鉴定等各个环节还有待进一步优化。

按照青枯菌侵染进程和致病特点(Chun-Mei et al., 2002),将这 384 个蛋白从糖蛋白、信号肽蛋白和跨膜蛋白等七个方面进行了分类,得到致病性青枯菌菌株分泌蛋白组分的详细信息。这其中 17 个蛋白质前人研究报道可能与青枯菌的致病性相关,其中包括草杆菌素蛋白酶 A3RTC4 和 B2UBR3 两种(魏艺聪等, 2009)、Lrr-gala 家族 3 型分泌蛋白 B5S4F6 (Mukaihara and Tamura, 2009)、popf1 分泌蛋白 Q8XPT2 (Meyer et al., 2006)、蛋白 PopA (Kanda et

al., 2008)、蛋白B5SAX2 (Sun et al., 2009)、外膜蛋白OmpW和OmpA/MotB四种(Sun et al., 2009)、蛋白PilA和PilT (Kang et al., 2002)、蛋白FtsN、异青霉素N 差向异构酶(Derouaux et al., 2008)、血溶素样蛋白Q8XT20/Q8Y2T5/Q8Y377/Q8Y378三种(Blum et al., 1995)。这些结果说明使用shotgun蛋白质组学技术可有效的分析青枯菌分泌蛋白, 具有可行性和有效性。同时在这384个蛋白中, 有327个迄今为止尚无文献报道与青枯菌的致病性相关, 有40个为未知蛋白。这个结果一方面的原因可能是因为目前关于青枯菌分泌蛋白的研究较少; 另一方面是由于shotgun蛋白质组学技术较其他的传统蛋白检测手段例如免疫杂交等或者传统的基于凝胶系统的2D技术具有更高的检测水平, 可以检测到更多的低丰度蛋白质(Listgarten and Emili, 2005)。这些首次鉴定到的蛋白和未知蛋白都为烟草青枯菌致病性研究及烟草青枯菌蛋白组数据库的建立积累了重要资料, 为进一步阐述烟草青枯菌的致病机理提供了实验数据和理论基础, 这也是我们下一步研究工作的重点。

3材料与方法

3.1 菌种培养

供试菌株为茄青枯雷尔氏菌(*Ralstonia solanacearum*)。根据细菌的生理生化特性, 属于为Race1 (Biovar 3)品种, 来自中国农科院。本供试菌株为杆状, 革兰氏染色阴性, 参考陈晓敏的方法在TTC平板上30℃下划线培养(陈晓敏等, 2000)。48 h后, 可出现两种不同的菌落: 一种是中心呈粉红色或浅色的稀液状、较宽的白边、不规则形或近圆形、流动性强、致病力强的野生菌落; 另一种是致病力弱或丧失致病力的变异菌落, 它们呈圆形、光滑、干燥、较扁平、玫瑰红色、白边很窄(李文溶和段道雄, 1988)。挑取致病菌菌株(virulent strain)继续培养。

3.2 样品制备

致病性青枯菌分别接种于分泌培养基(10 克酸水解酪素, 蒸馏水 1 000 mL, PH7.0~7.46) (陈晓敏等, 2000)中, 30℃振荡培养(200 转/min) 48 小时, 当培养液 OD_{600nm}=0.6 时将培养液以 6000 转/min 离心, 收集上清。上清用 Amicon Ultra-15(截留分子

量 5 K)超滤装置浓缩至 1 mL 后加入 5 mL 预冷的丙酮(含 1%二硫苏糖醇)沉淀, 沉淀产物冷冻干燥后加入含 8M 尿素, 150 mM 甲基氨基甲烷, 蛋白酶抑制剂的裂解液(pH8.5), 4℃放置 30 min 后以 12 000 转/min 于 4℃离心 2 小时, 取上清, 上清用 GE-2D 除盐试剂盒(GE)除盐后以 Bradford 法定量(Bio-Rad)。

3.3 SDS-聚丙烯酰胺凝胶电泳以及酶解

致病菌株提取的分泌蛋白样品加入二硫苏糖醇至终浓度 10 mmol/L, 放置于 37℃ 2.5 h 后加入碘乙酰胺至终浓度 50 mmol/L, 室温避光反应 40 min。还原和烷基化反应后的样品进行 SDS-PAGE 垂直电泳(12.5T%), 每个泳道各上样 20 μg。电泳条件为: 15 mA 电泳 20 min 后以 30 mA 电流直至溴酚蓝前沿迁移至底端。SDS-PAGE 胶经考马斯亮蓝 R-350 (GE)染色后每组样品分成 5 份, 每份条带首先用 50 mmol/L 碳酸氢胺/30%乙腈脱色, 再用 100%乙腈处理 5 min, 干燥后加入 50 mmol/L 碳酸氢胺, 10 min 后弃去, 再加入 100%乙腈, 5 min 后弃去, 干燥后加入以蛋白:酶=50:1 的比例加入胰蛋白酶(Promega Biotech Co., Madison, WI, USA), 37℃ 反应 12 h。酶解产物用 0.1% TFA/60%乙腈超声抽提后冷冻干燥, 酶解样品于-70℃保存待用。

3.4 LC-MS/MS

酶解样品复溶于0.1%甲酸后用MDLC进行反相C18-HPLC (150×100 mm Column technology Inc., Fremont, CA)分离, 泵流速65微升/分钟。经由分流器, 柱出口流速为2 μL/min。流动相A为0.1%甲酸/水, 流动相B为84%乙腈/0.1%甲酸/水。梯度设置如下: 4%~50% B 105 min, 100% B维持15 min。RP-HPLC柱出口经由电喷雾接口(ESI)与LTQ线形离子阱质谱相连, 具体设置如下: 加热毛细管温度为160℃, ESI needle 电压为3.0 kV, 碰撞能量为35.0%。使用AGC模式采集信号, 毛细管与四级杆等参数由调谐程序自动优化, 采集方式为1个全扫描(profile模式)后对全扫描的5个相对最强离子进行MS/MS扫描, 其中有动态排除设置(重复2次, 重复间隔0.5 min, 排除间隔1.5 min)。

3.5 LTQ结果分析

LTQ原始文件经由BioWorks 3.0软件组自带的Sequest Cluster进行查库分析, 数据库来自swissprot (<http://www.uniprot.org/taxonomy>, Taxon identifier: 48736 proteome set for *Ralstonia* 5/27/2009 released), 查库参数简要为母离子宽容度3 Da, 碎片离子宽容度为0.8 Da, 固定修饰设置半胱氨酸(+57.02 Da), 动态修饰设置甲硫氨酸氧化(+57.02 Da), 胰蛋白酶最多允许两个内部酶切位点。利用BioWorks生成*Ralstonia*正反库, 筛选查库结果假阳性率为0.05%, 并且含有两个以上唯一肽段的蛋白, 去除冗余数据后进行统计学分析。

作者贡献

宋浩、丁伟、沙伟、刘翀、周燕是本研究的实验设计和实验研究的执行人; 丁伟和宋浩及沙伟完成数据分析, 论文初稿的写作; 宋浩和丁伟参与实验设计, 试验结果分析; 陈薇是项目的构思者及负责人, 指导实验设计, 数据分析, 论文写作与修改。全体作者都阅读并同意最终的文本。

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参考文献

- Blum G, Falbo V, Caprioli A, and Hacker J, 1995, Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae and alpha-hemolysin form the pathogenicity island II of the uropathogenic *Escherichia coli* strain J96, *FEMS Microbiol Lett*, 126(2): 189-195
- Cao P, Zhang H.M., Zhao X.L., Li M., and Chang W.R., 2009, The crystallography study of some plant pathogens lilac pseudomonads effect protein, *Shengwu Wuli Xuebao (Acta Biophysica sinica)*, 25: 315 (曹鹏, 张红梅, 赵学琳, 李梅, 常文瑞, 2009, 几种植物致病菌丁香假单胞杆菌效应蛋白的晶体学研究, *生物物理学报*, 25: 315)
- Chen X.M., Hu F.P., and Wu Y.R., 2000, Pathotypes and biotypes of *Ralstonia solanacearum* isolated from bacterial wilt of peanut in Fujian Province, *Fujian Nongye Daxue Xuebao (Journal of Fujian Agricultural University)*, 29(4):

470-473(陈晓敏, 胡方平, 吴燕榕, 2000, 福建省花生青枯病菌致病型及生物型的测定, *福建农业大学学报*, 29(4): 470-473)

- Chun-Mei L., Ian B., John M., Conrad S., Tristan B., Martin R, and Suvi T, 2002, The Hrp pilus of *Pseudomonas syringae* elongates from its tip and acts as a conduit for translocation of the effector protein HrpZ, *EMBO*, 21(8): 1909-1915
- Dahal D., Pich A., Braun H.P., and Wydra K., 2010, Analysis of cell wall proteins regulated in stem of susceptible and resistant tomato species after inoculation with *Ralstonia solanacearum*: a proteomic approach, *Plant Mol Biol*, 73(6): 643-658
- Dangl J.L., and Jones J.D., 2001, Plant pathogens and integrated defence responses to infection, *nature*, 411: 826-833
- Denny T.P., Carney B.F., and Schell M.A., 1990, Inactivation of multiple virulence genes reduces the ability of *Pseudomonas solanacearum* to cause wilt symptoms, *Mol Plant-Microbe Interact*, 3: 293-300
- Derouaux A., Wolf B., Fraipont C., Breukink E., Nguyen-Distèche M., and Terrak M., 2008, The monofunctional glycosyltransferase of *Escherichia coli* localizes to the cell division site and interacts with penicillin-binding protein 3, FtsW, and FtsN, *J. Bacteriol*, 190(5): 1831-1834
- Fujiwara A., Kawasaki T., Usami S., Fujie M., and Yamada T., 2008, Genomic characterization of *Ralstonia solanacearum* phage phiRSA1 and its related prophage (phiRSX) in strain GMI1000, *J. Bacteriol*, 190(1): 143-156
- Genin S, and Boucher C, 2002, *Ralstonia solanacearum*: secrets of a major pathogen unveiled by analysis of its Genome, *Mol. Plant Pathol.*, 3: 111-318
- Genin S., 2002, *Ralstonia solanacearum*: Secrets of a major pathogen unveiled by analysis of its genome, *Molecular Plant Pathology*, 3: 111-118
- Guidot A., Coupat B., Fall S., Prior P., and Bertolla F., 2009, Horizontal gene transfer between *Ralstonia solanacearum* strains detected by comparative genomic hybridization on microarrays, *ISME J.*, 3(5): 549-562
- Guidot A., Prior P., Schoenfeld J., Carrère S., Genin S., and Boucher C., 2007, Genomic structure and phylogeny of the plant pathogen *Ralstonia solanacearum* inferred from gene distribution analysis, *J. Bacteriol*, 189(2): 377-387
- Hammond-Kosack K.E., and Parker J.E., 2003, Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding, *14(2): 177-193*

- Huang H.E., Liu C.A., Lee M.J., Kuo C.G., Chen H.M., Ger M.J., Tsai Y.C., Chen Y.R., Lin M.K., and Feng T.Y., 2007, Resistance enhancement of transgenic tomato to bacterial pathogens by the heterologous expression of sweet pepper ferredoxin-I protein, *Phytopathology*, 97(8): 900-906
- Huang J.L., Wu J.Z., Xiao C.G., Li C.J., and Wang G.X., 2007, Analysis of signal peptides of the secreted proteins in *Ralstonia solanacearum* GMI1000, *Yi Chuan*, 29(11): 1409-1416
- Jiang H., Liao B., Ren X., Lei Y., Mace E., Fu T., and Crouch J.H., 2007, Comparative assessment of genetic diversity of peanut (*Arachis hypogaea* L.) genotypes with various levels of resistance to bacterial wilt through SSR and AFLP analyses, *J. Genet Genomics*, 34(6): 544-554
- Kanda A., Ohnishi K., Kiba A., and Hikichi Y., 2008, Implication of C-terminal mutation of PopA of *Ralstonia solanacearum* strain OE1-1 in suppression of bacterial wilt, *Plant Pathology*, 58(1): 159-169
- Kang Y., Huang J., and Mao G., et al. Dramatically reduced virulence of mutants of *R. solanacearum* defective in export of extracellular proteins across the outer membrane, *Mol. Plant Microbe Interact.*, 1994, 7: 370-377
- Kang Y., Kim J., Kim S., Kim H., Lim J.Y., Kim M., Kwak J., Moon J.S., and Hwang I., 2008, Proteomic analysis of the proteins regulated by HrpB from the plant pathogenic bacterium *Burkholderia glumae*, *Proteomics*, 8(1): 106-121
- Kang Y., Liu H., Genin S., Schell M.A., and Denny T.P., 2002, *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence, *Mol. Microbiol.*, 46(2): 427-437
- Koster M., Bitter W., and Tommassen J., 2000, Protein secretion mechanisms in gram-negative bacteria, *International Journal of Medical Microbiology*, 290: 325-331
- Li L.Z., Xie C.H., and Liu J., 2005, Molecular Biology and Pathogenicity of *Ralstonia solanacearum*, *Zhongguo Malingshu (Chinese Potato Journal)*, 19(5): 290-294 (李林章, 谢从华, 柳俊, 2005, 茄科雷尔氏菌(*Ralstonia solanacearum*)分子生物学基础及其致病机制, *中国马铃薯*, 19(5): 290-294)
- Listgarten J., and Emili A., 2005, Statistical and computational methods for comparative proteomic profiling using liquid chromatography-tandem mass spectrometry, *Mol Cell Proteomics*, 4(4): 419-434
- Li W.R., and Duan Q.X., 1988, Study of pathogenicity of *Ralstonia solanacearum* to peanut, *Zhongguo Youliao (China Oil)*, 4:(1-4) (李文溶, 段迺雄, 1988, 花生青枯菌致病性的研究, *中国油料*, (4): 1-4)
- Matallana-Surget S., Leroy B., and Wattiez R., 2010, Shotgun proteomics: concept, key points and data mining, *Expert Rev. Proteomics*, 7(1): 5-7
- Meyer D., Cunnac S., Guéron M., Declercq C., Van Gijsegem F., Lauber E., Boucher C., and Arlat M., 2006, PopF1 and PopF2, two proteins secreted by the type III protein secretion system of *Ralstonia solanacearum*, are translocators belonging to the HrpF/NopX family, *J. Bacteriol.*, 188(13): 4903-4917
- Monchy S., Benotmane M.A., Wattiez R., van Aelst S., Auquier V., Borremans B., Mergeay M., Taghavi S., van der Lelie D., and Vallaey T., 2006, Transcriptomic and proteomic analyses of the pMOL30-encoded copper resistance in *Cupriavidus metallidurans* strain CH34, *Microbiology*, 152(Pt 6): 1765-1776
- Mota L.J., and Cornelis., 2005, The bacterial injection kit: type III secretion systems, *Ann Med.*, 37(4): 234-249
- Mukaihara T., Tamura N., 2009, Identification of novel *Ralstonia solanacearum* type III effector proteins through translocation analysis of hrpB-regulated gene products, *Microbiology*, 155(Pt7): 2235-2244
- Orans J., Johnson M.D., Coggan K.A., Sperlazza J.R., Heiniger R.W., Wolfgang M.C., and Redinbo M.R., 2010, Crystal structure analysis reveals *Pseudomonas* PilY1 as an essential calcium-dependent regulator of bacterial surface motility, *Proc. Natl. Acad. Sci. USA*, 107(3): 1065-1070
- Orgambide G, Montrozier H, Servin P, Roussel J, Trigalet-Demery D, and Trigalet A, 1991, High heterogeneity of the exopolysaccharides of *Pseudomonas solanacearum* strain GMI 1000 and the complete structure of the major polysaccharide, *J Biol Chem*, 266(13): 8312-8321
- Poueymiro M., Genin S., 2009, Secreted proteins from *Ralstonia solanacearum*: a hundred tricks to kill a plant, *Curr. Opin. Microbiol.*, 12(1): 44-52
- Remenant B., Coupat-Goutaland B., Guidot A., Cellier G, Wicker E., Allen C., Fegan M., Pruvost O., Elbaz M., Calteau A., Salvignol G, Mornico D., Mangenot S., Barbe V., and Médigue C., 2010, Prior P., Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence, *BMC Genomics*, 11: 379

- Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L, Chandler M, Choisine N, Claudel-Renard C, Cunnac S, Demange N, Gaspin C, Lavie M, Moisan A, Robert C, Saurin W, Schiex T, Siguier P, Thébault P, Whalen M, Wincker P, Levy M, Weissenbach J, and Boucher CA, 2002, Genome sequence of the plant pathogen *Ralstonia solanacearum*, *Nature*, 415: 497-502
- Song H., Sha W., Ding W., Liu C., Zhou Y., and Chen W., 2010, The Application of Label-free Quantification Proteomics in Tobacco *Ralstonia solanacearum* Pathogenicity Research, *Fenzi Zhiwu Yuzhong (Molecular Plant Breeding)*, 8(3): 1-10 (宋浩, 沙伟, 丁伟, 刘翀, 周燕, 陈薇, 2010, 非标记定量蛋白质组学在烟草青枯菌致病性研究中的应用, *分子植物育种*, 8(3): 1-10)
- Sun W., Roland K.L., Branger C.G., Kuang X., Curtiss R., 2009, The role of *relA* and *spoT* in *Yersinia pestis* KIM5 pathogenicity, *PLoS One*, 4(8): e6720
- Wei Y.C., Wang Z.H., and Lu G.D., 2009, Bioinformatics Analysis of Subtilases in *M. grisea* and Secretion Detection of MGG_07965.6, *Zhongguo Nongxue Tongbao (CHINESE AGRICULTURAL SCIENCE BULLETIN)*, 25(24): 50-55 (魏艺聪, 王宗华, 鲁国东, 2009, 稻瘟病菌Subtilases家族生物信息学分析及其中-蛋白的分泌性检验, *中国农学通报*, 25(24): 50-55)
- Xia Q.C., and Zeng R., eds., 2004, *Protein Chemistry and Proteomics*, Science Press, Beijing, China, pp. 278 (夏其昌, 曾嵘, 编著, 2004, *蛋白质化学和蛋白质组学*, 科学出版社, 中国, 北京, pp. 278)
- Yang Y.C., Zhou Q.M., and Zhu L.S., 2006, Heredity and RAPD markers analysis of resistance gene to tobacco bacterial wilt, *Zhongguo Yancao Xuebao (Chinese Tobacco Science)*, 12(02): 38-42 (杨友才, 周清明, 朱列书, 2006, 烟草青枯病抗性基因的遗传分析及RAPD标记, *中国烟草学报*, 12(02): 38-42)
- Zhang G, Chen M., Li L., Xu Z., Chen X., Guo J., and Ma Y., 2009, Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco, *J. Exp. Bot.*, 60(13): 3781-96



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表 1 LTQ/MS/MS 鉴定蛋白质结果

Table 1 Identification of proteins based on MS/MS spectra from the LTQ

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
B2U705	35246.63	6.54	Malate dehydrogenase
B2U8L3	14574.91	10.42	50S ribosomal protein L19
B2U9U5	15329.65	5.91	Nucleoside diphosphate kinase
B2UCV5	11302.07	10.05	50S ribosomal protein L21
B2UEK2	18227.31	10.16	30S ribosomal protein S5
B2UEK9	13351.65	10.27	50S ribosomal protein L14
B2UEL8	22931.31	9.91	50S ribosomal protein L4
B2UGJ5	13450.82	11.77	50S ribosomal protein L20
B2UGV1	55351.17	5.87	ATP synthase subunit alpha
P58597	40633.49	8.54	EPS I polysaccharide export outer membrane protein epsA
P58601	40960.46	8.93	Pectinesterase
Q8XGZ0	43167.4	5.43	Elongation factor Tu
Q8XT38	60732.97	8.11	Periplasmic trehalase
Q8XU74	55363.18	6.01	ATP synthase subunit alpha
Q8XV12	23179.49	9.89	50S ribosomal protein L3
Q8XV13	22948.34	9.91	50S ribosomal protein L4
Q8XV17	11957.92	10.19	50S ribosomal protein L22
Q8XV22	13351.65	10.27	50S ribosomal protein L14
Q8XV29	18194.32	10.16	30S ribosomal protein S5
Q8XV37	23142.39	10.13	30S ribosomal protein S4
Q8XVK8	11274.05	9.99	50S ribosomal protein L21
Q8XXW5	35422.77	6.25	Malate dehydrogenase
Q8XZ26	13480.81	11.55	50S ribosomal protein L20
Q8Y033	15316.65	5.91	Nucleoside diphosphate kinase
Q8Y099	31316.02	5.9	Dihydrodipicolinate synthase
Q8Y0B5	45711.75	4.8	Enolase
Q8Y0J1	8713.71	4.06	Acyl carrier protein 1
Q8Y0V7	14601.94	10.42	50S ribosomal protein L19
Q8Y3C6	52342.53	5.35	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B
Q9RBS0	33151.59	4.38	Protein popA1
A3RNR7	20858.51	9.39	Uncharacterized protein
A3RPA2	20318.67	11.18	50S ribosomal protein L19
A3RPK9	32611.41	8.58	Phytoene synthase
A3RPV4	48873.64	7.8	Alkaline phosphatase
A3RQ41	17027.62	9.59	PilA
A3RQA4	24003.68	10.06	uncharacterized protein
A3RQV4	43977.42	6.05	Isopenicillin N epimerase
A3RRM1	59078.28	6	Acetyl-CoA:acetoacetyl-CoA transferase alpha subunit
A3RRN0	32650.51	9.07	Glutamate/aspartate-binding protein
A3RRQ8	41757.53	5.95	Leucine-, isoleucine-, valine-, threonine-, and alanine-binding protein
A3RRV4	24801.54	7.18	Uncharacterized protein
A3RRW1	21232.65	9.37	Uncharacterized protein
A3RRX2	17795	6.69	Superoxide dismutase [Cu-Zn]
A3RS71	11321.12	10.16	50S ribosomal protein L21
A3RSB4	18476.13	4.81	Bacterioferritin
A3RTC4	53087.77	5.51	Extracellular subtilisin-like protease

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
A3RTF1	38166.82	6.4	PilT
A3RTQ8	37958.77	6.75	Uncharacterized protein
A3RTR4	60052.62	9.49	Cyclohexanone monooxygenase
A3RTX1	33783.86	8.38	Uncharacterized protein
A3RU12	25951.82	5.73	Ribulose-phosphate 3-epimerase
A3RU89	57332.55	5.12	60 kDa chaperonin
A3RU95	54229.42	5.23	Uncharacterized protein conserved in bacteria
A3RUG8	28299.66	4.84	FliC
A3RUM0	37826.73	5.81	Purine nucleoside permease
A3RUT9	37362.34	9.35	Phosphate-binding protein
A3RVQ4	35442.8	6.55	Malate dehydrogenase
A3RVT0	20735.25	8.78	Uncharacterized protein
A3RVY2	13351.65	10.27	50S ribosomal protein L14
A3RVY9	18223.31	10.16	SSU ribosomal protein S5P
A3RVZ7	23226.56	10.13	SSU ribosomal protein S4P
A3RW48	18416.45	5.87	Peptidyl-prolyl cis-trans isomerase
A3RW57	61951.52	8.59	Transmembrane methyl-accepting chemotaxis protein
A3RW82	45857.88	4.85	Enolase
A3RWC1	13480.81	11.55	50S ribosomal protein L20
A3RWL8	27353.29	8.97	ABC transporter amino acid-binding protein
A3RWZ4	25140.61	9.77	FtsN
A3RX63	74336.04	6.43	Exo-poly-alpha-D-galacturonidase
A3RXG1	52402.17	5.48	Glutamine synthetase
A3RXH0	57181.37	5.75	Tek
A3RYC9	60229.02	6.16	Medium-chain-fatty-acid--CoA ligase
A3RYY1	50505.89	6.09	Aldehyde dehydrogenase
A3RZ67	41184.33	5.78	Glycerophosphoryl diester phosphodiesterase
A3RZA6	43095.3	5.5	Elongation factor Tu
A3RZI0	39137.78	10	Integral membrane protein
A3RZU5	16097.4	8.52	VirK protein
A3S031	30811.65	9.57	Uncharacterized protein
A3S0Z9	33545.44	9.14	Glutamate/aspartate-binding protein
A7C8V2	23175.43	4.25	Periplasmic ligand-binding sensor protein
A7C9I6	25711.12	9.51	Flagellar L-ring protein
A7C9W8	49496.8	6.1	Nicotinate phosphoribosyltransferase
A7C9Y2	56226.31	9.08	Membrane protein involved in aromatic hydrocarbon degradation
A7CAJ2	46059.25	9.38	Transposase, mutator type
A7CAK6	55549.41	5.92	Oxygen-independent coproporphyrinogen III oxidase
A7CBV3	24830.3	10.22	Sporulation domain protein
A7CC25	10611.98	10.77	Uncharacterized protein
A7CC87	46145.31	9.31	Transposase, mutator type
A7CCM5	48364.07	8.23	Alkaline phosphatase
A7CD19	55351.17	5.87	ATP synthase subunit alpha
A7CDP0	54381.68	6.98	Peptidase S8 and S53, subtilisin, kexin, sedolisin
A7CDR5	38193.81	6.36	Twitching motility protein
A7CDV4	78066	8.56	ATP-dependent DNA helicase RecG
A7CE75	11302.07	10.05	50S ribosomal protein L21
A7CEA3	38058.07	6.12	Purine nucleoside permease

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
A7CEI5	22816.19	9.39	Transposase, mutator type
A7CES3	28667.13	9.38	Transport-associated protein
A7CF52	32972.78	9.16	Extracellular solute-binding protein, family 3
A7CFB0	32200.41	9.65	OmpW family protein
A7CFM1	57548.92	5.14	60 kDa chaperonin
A7CG74	45712.29	7.88	Lipoprotein releasing system, transmembrane protein, LolC/E family
A7CGE7	11783.62	5.3	Thioredoxin
A7CGH0	15329.65	5.91	Nucleoside-diphosphate kinase
A7CGZ1	33164.64	5.06	Uncharacterized protein
A7CH37	18765.25	8.69	Uncharacterized protein
A7CH58	57305.07	4.73	Uncharacterized protein
A7CHK7	25924.8	5.73	Ribulose-phosphate 3-epimerase
A7CHP0	74707.77	5.62	ATP-dependent endonuclease of the OLD family-like protein
A7CI28	18227.31	10.16	Ribosomal protein S5
A7CI35	13351.65	10.27	50S ribosomal protein L14
A7CI45	22931.31	9.91	Ribosomal protein L4/L1e
A7CI48	43624.95	5.42	Elongation factor Tu
A7CI90	120197.9	6.37	DNA polymerase III, alpha subunit
A7CIZ8	35219.61	6.25	Malate dehydrogenase
A7CJ57	21447.93	9.3	Uncharacterized protein
A7CJ72	55134.09	5.71	Aldehyde dehydrogenase (NAD(+))
A7CJA3	33488.4	8.88	Extracellular solute-binding protein, family 3
A7CK05	23676.72	9.89	Uncharacterized protein
A7CK23	11959.89	8.96	Conserved hypothetical signal peptide protein
A7CK73	27042.03	8.99	Phosphatidylserine/phosphatidylglycerophosphate/ cardiolipin synthases and related enzymes-like protein
A7CK97	27559.27	5.49	Uncharacterized protein
A7CKD8	65521.07	8.12	Uncharacterized protein
A7CKG4	17797.44	10.07	ISRSO7-transposase protein (Fragment)
A7CKK6	58472.27	9.72	Uncharacterized protein
A7CKQ4	6243.21	4.85	Uncharacterized protein
A7CLD6	11921.98	9.48	Conserved hypothetical signal peptide protein
A7CLP7	18319.76	8.81	Uncharacterized protein
A7CM53	23316.48	9.46	OmpA/MotB domain protein
A7CM55	20247.19	9.32	Signal peptide protein
A7CM90	14575.9	10.31	50S ribosomal protein L19
A7CMH9	129379.94	5.8	DNA polymerase III, alpha subunit
A7CMW3	9020.53	10.79	50S ribosomal protein L20
A7CN35	60045.75	5.84	AMP-dependent synthetase and ligase
B2U6M6	57402.7	5.08	60 kDa chaperonin
B2U773	50311.12	5.81	Alkaline phosphatase
B2U855	37968.04	6.44	Purine nucleoside permease
B2U879	23316.48	9.46	OmpA/MotB domain protein
B2U881	20220.17	9.32	Signal peptide protein
B2U907	129303.88	5.77	DNA polymerase III, alpha subunit
B2U9G4	33529.51	9	Extracellular solute-binding protein family 3
B2U9J4	55134.09	5.71	Aldehyde Dehydrogenase

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
B2U9M7	21373.86	9.3	Uncharacterized protein
B2U9S2	11783.62	5.3	Thioredoxin
B2UBR3	54322.54	6.21	Peptidase S8 and S53 subtilisin kexin sedolisin
B2UBU1	38194.84	6.36	Twitching motility protein
B2UC23	59934.72	5.99	AMP-dependent synthetase and ligase
B2UCB7	37306.54	9.21	Porin Gram-negative type
B2UD09	25894.77	5.73	Ribulose-phosphate 3-epimerase
B2UDF2	23676.72	9.89	Uncharacterized protein
B2UDH0	11959.89	8.96	Conserved hypothetical signal peptide protein
B2UDS9	35459.23	5.94	Uncharacterized protein
B2UDV9	24871.38	10.1	Sporulation domain protein
B2UFJ2	32956.79	9.16	Extracellular solute-binding protein family 3
B2UFP6	32261.53	9.62	OmpW family protein
B2UG55	28753.24	9.41	Transport-associated
B2UG76	55493.32	6.07	Oxygen-independent coproporphyrinogen III oxidase
B2UH93	23175.43	4.25	periplasmic ligand-binding sensor protein
B2UI11	31966.86	9.54	Transcriptional regulator, AraC family
B2UIL8	65521.07	8.12	uncharacterized protein
B2UJ29	120197.9	6.37	DNA polymerase III, alpha subunit
B2UJH3	47730.61	8.42	Membrane protein involved in aromatic hydrocarbon degradation
B2UJI8	49496.8	6.1	Nicotinate phosphoribosyltransferase
B2UJW8	25711.12	9.51	Flagellar L-ring protein
B5RV70	17448.61	6.69	Superoxide dismutase [Cu-Zn]
B5RV81	21232.65	9.37	Bacterial outer membrane protein
B5RVM9	11321.12	10.16	50S ribosomal protein L21
B5RW01	38166.82	6.4	Twitching mobility protein transport fimbria
B5RW28	53293.92	5.25	Protease protein
B5RWH7	25951.82	5.73	Ribulose-phosphate 3-epimerase protein
B5RWU4	12829.71	10.94	Hypothetical 30S ribosomal protein s4 (Partial sequence c terminus) (Fragment)
B5RWW2	17216.12	10.08	30S ribosomal protein s5
B5RWW9	13351.65	10.27	50S ribosomal protein L14
B5RXC1	24003.68	10.06	Amino-acid transmembrane protein
B5RXI8	28332.8	8.62	Periplasmic or secreted lipoprotein
B5RZ16	50436.02	5.67	Uncharacterized protein
B5RZE3	38059.84	6.75	Uncharacterized protein
B5RZE9	59932.42	9.49	Monooxygenase protein
B5RZG3	13285.06	8.33	Uncharacterized protein
B5RZI2	45857.88	4.85	Enolase
B5RZL1	62616.29	8.59	Methyl-accepting chemotaxis transducer protein
B5RZL9	18416.45	5.87	Peptidyl-prolyl cis-trans isomerase
B5RZQ9	20858.51	9.39	Lipid a deacylase protein
B5S060	41820.96	9.83	Outer membrane protein
B5S0M8	50659.17	6.22	Betaine aldehyde dehydrogenase (Badh) protein
B5S0X9	74443.2	6.57	Exo-poly-galacturonosidase protein
B5S0Y5	60227.05	6.16	Acyl-coa synthetase protein
B5S171	15274.57	5.91	Nucleoside diphosphate kinase (Ndk) (Ndp kinase) (Nucleoside-2-pkinase) protein

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B5S1C1	52402.17	5.48	Glutamine synthetase
B5S1T9	15551.8	8.52	Uncharacterized protein
B5S1V8	33545.44	9.14	Amino-acid-binding periplasmic protein (Pbp)
B5S209	5346.07	9.1	Uncharacterized protein
B5S2F7	35452.84	6.55	Malate dehydrogenase protein
B5S2Q5	36248.14	9.28	Phosphate-binding periplasmic protein (Pbp)
B5S2W1	13480.81	11.55	50S ribosomal protein L20
B5S395	32669.48	8.58	Phytoene synthase protein
B5S3M0	39148.85	10	Uncharacterized protein
B5S3Q6	52061.67	6.19	Serine/threonine-specific protein phosphatase and bis(5-nucleosyl)- tetrphosphatase;
B5S3Y4	27383.31	8.97	Substrate-binding periplasmic protein (Pbp)
B5S4F6	108178.79	6.31	Lrr-gala family type III effector protein (Gala 2)
B5S4M4	22508.42	9.41	Lipoprotein
B5S4N3	102205.59	9.08	Uncharacterized protein
B5S566	53726.37	5.24	Tek protein
B5S5B0	27656.98	9.42	Cog3687, predicted metal-dependent hydrolase protein
B5S6C7	8713.71	4.06	Acyl carrier protein 1 (Acp 1)
B5S6G3	35872.91	6.48	Cysteine sulfinate desulfinate / cysteine desulfurase protein (Partial sequencen terminus) (Fragment)
B5S6P9	15377.91	10.37	50S ribosomal protein L19
B5S6W8	17716.98	4.59	Phage capsid-related protein
B5S701	15664.86	4.69	Flagellin (Partial sequence n terminus) protein
B5S7I1	49700.56	5.37	Alkaline phosphatase lipoprotein
B5S7I2	48889.61	7.21	Alkaline phosphatase protein
B5S7X2	25159.66	9.88	Cell division protein ftsn
B5S840	18476.13	4.81	Bacterioferritin
B5S8G3	59096.29	6	Coenzyme a transferase; protein
B5S8H3	32650.51	9.07	Amino-acid-binding periplasmic protein (Pbp)
B5S8J7	41335.55	5.93	Glycerophosphoryl diester phosphodiesterase, periplasmic protein
B5S8R6	16956.54	9.59	Type 4 fimbrial pilin protein
B5S8Z2	57577.84	5.12	60 kDa chaperonin
B5S9F0	37800.65	5.81	Purine nucleoside permease protein
B5SA46	28299.66	4.84	Flagellin protein
B5SAX2	54229.42	5.23	Hypothetical rela/spot domain protein
B5SBY2	27353.29	8.97	Substrate-binding periplasmic protein (Pbp)
B5SC50	71125.95	6.15	Serine protease protein
B5SCZ8	53840.52	5.36	Tek protein
B5SD37	27697.05	9.42	Cog3687, predicted metal-dependent hydrolase protein
B5SDR8	39137.78	10	Uncharacterized protein
B5SDV8	25140.61	9.77	Cell division protein ftsn
B5SDY7	18476.13	4.81	Bacterioferritin
B5SEK3	48873.64	7.8	Alkaline phosphatase protein
B5SER5	24003.68	10.06	Amino-acid transmembrane abc transporter protein
B5SF16	15377.91	10.37	50S ribosomal protein L19
B5SFG9	33545.44	9.14	Amino-acid-binding periplasmic protein (Pbp)

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
B5SFR4	15551.8	8.52	Uncharacterized protein
B5SG51	59078.28	6	Coenzyme a transferase; protein
B5SG62	32650.51	9.07	Amino-acid-binding periplasmic protein (Pbp)
B5SG86	41184.33	5.78	Glycerophosphoryl diester phosphodiesterase, periplasmic protein
B5SGF7	37826.73	5.81	Purine nucleoside permease protein
B5SGQ4	17027.62	9.59	Type 4 fimbrial pilin protein
B5SGX6	57332.55	5.12	60 kDa chaperonin
B5SHN2	53087.77	5.51	Protease protein
B5SHR2	38166.82	6.4	Twitching mobility protein transport fimbria
B5SHZ9	21232.65	9.37	Bacterial outer membrane protein
B5SII2	17795	6.69	Superoxide dismutase [Cu-Zn]
B5SIE7	11321.12	10.16	50S ribosomal protein L21
B5SIR5	35442.8	6.55	Malate dehydrogenase protein
B5SIW2	25951.82	5.73	Ribulose-phosphate 3-epimerase protein
B5SJC5	36248.14	9.28	Phosphate-binding periplasmic protein (Pbp)
B5SJJ3	23226.56	10.13	30s ribosomal protein s4
B5SJJ1	17230.15	10.08	30s ribosomal protein s5
B5SJJ7	41473.37	5.4	Elongation factor Tu (Fragment)
B5SJS3	13480.81	11.55	50S ribosomal protein L20
B5SK08	60052.62	9.49	Monoxygenase protein
B5SK14	37958.77	6.75	Uncharacterized protein
B5SK83	43977.42	6.05	Aminotransferase, class v; protein
B5SKC5	32611.41	8.58	Phytoene synthase protein
B5SKG7	60229.02	6.16	Acyl-coa synthetase protein
B5SKP6	31153.81	6.44	Periplasmic or secreted lipoprotein
B5SKY3	74336.04	6.43	Exo-poly-galacturonidase protein
B5SL32	50505.89	6.09	Betaine aldehyde dehydrogenase (Badh) protein
B5SLU8	52402.17	5.48	Glutamine synthetase
B5SM02	15274.57	5.91	Nucleoside diphosphate kinase (Ndk) (Ndp kinase) (Nucleoside-2-pkinase) protein
B5SM49	18416.45	5.87	Peptidyl-prolyl cis-trans isomerase
B5SM58	61951.52	8.59	Methyl-accepting chemotaxis transducer protein
B5SM86	45857.88	4.85	Enolase
B5SMF6	8713.71	4.06	Acyl carrier protein 1 (Acp 1)
B7ZJG6	32650.15	4.43	Type III effector protein
C0SPN3	31996.46	4.4	Hrp-secreted outer protein
C0SPP3	243440.92	8.86	Type III effector protein
O30592	37000.06	5.29	CrpB
Q0PHK7	57743.31	5.25	BtxP
Q0Q2Y4	11205.93	4.36	Flagellin (Fragment)
Q0Q2Y5	11177.88	4.36	Flagellin (Fragment)
Q0Q2Y8	11173.94	4.36	Flagellin (Fragment)
Q0Q2Z3	11121.83	4.24	Flagellin (Fragment)
Q0Q2Z4	11117.84	4.36	Flagellin (Fragment)
Q0Q2Z6	11120.84	4.36	Flagellin (Fragment)
Q0Q2Z8	11147.86	4.36	Flagellin (Fragment)
Q0Q318	11145.9	4.14	Flagellin (Fragment)

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
Q0Q320	11175.91	4.36	Flagellin (Fragment)
Q0Q324	11144.91	4.24	Flagellin (Fragment)
Q0Q326	11160.91	4.24	Flagellin (Fragment)
Q53241	74232.03	6.43	Exo-polygalacturonase PehB
Q6PW28	26868.97	7.11	regulatory protein
Q83UT8	24963.8	4.54	Flagellin (Fragment)
Q847Y7	24832.61	4.54	Flagellin (Fragment)
Q847Y8	24817.66	4.45	Flagellin (Fragment)
Q847Z3	25187.05	4.67	Flagellin (Fragment)
Q847Z5	25174.02	4.57	Flagellin (Fragment)
Q847Z6	25173.03	4.67	Flagellin (Fragment)
Q84IE8	32633.17	4.43	PopA
Q8XPI6	26128.68	10.41	Signal peptide protein
Q8XPQ7	56470.32	8.38	Catalase
Q8XPR8	49172.74	6.07	Uncharacterized protein
Q8XPT2	77284.97	5.82	Secreted protein popfl
Q8XPU8	23383.44	8.34	Lipoprotein
Q8XQI5	46749.91	6.98	Membrane-bound lytic murein transglycosylase b protein
Q8XQJ7	37130.71	5.68	Signal peptide protein
Q8XQM1	20781.39	9.2	Lipid a deacylase signal peptide protein
Q8XQP7	74633.67	5.58	Acid phosphatase protein
Q8XR13	53132.74	6.07	Transmembrane aldehyde dehydrogenase oxidoreductase protein
Q8XR28	113462.21	7.08	Cation efflux system transmembrane protein
Q8XR38	27108.17	7.86	Cog3687, predicted metal-dependent hydrolase protein
Q8XR59	57244.43	5.56	Tek signal peptide protein
Q8XR61	55095.8	4.87	Tek-related protein
Q8XRC8	252123.14	8.81	Type III effector protein (Skwp3)
Q8XRC9	11970.94	9.75	Hypothetical signal peptide protein
Q8XRE1	40714.47	6.51	Oxidoreductase, n-terminal; protein
Q8XRJ8	70761.66	6.12	Polygalacturonase
Q8XRS3	50908.88	6.68	Hypothetical twin-arginine translocation pathway signal harboring protein;
Q8XRS6	113436.23	9.07	Transmembrane protein
Q8XRT6	18455.42	5.8	Uncharacterized protein
Q8XRT9	22359.23	9.41	Lipoprotein
Q8XS78	69890.33	6.43	Serine protease protein
Q8XS79	13883.73	7.78	Signal peptide protein
Q8XSE4	81566.56	6.12	Aminopeptidase transmembrane protein
Q8XSJ1	38538.99	5.65	Ornithine cyclodeaminase protein
Q8XSJ3	27230.08	8.98	Substrate-binding periplasmic (Pbp) abc transporter protein
Q8XST5	28373.73	4.84	Flagellin protein
Q8XT20	70181.92	4.34	Hemolysin-type protein
Q8XT40	41927.46	6.98	Glycosyl hydrolase family 18 with carbohydrate binding domain transmembrane protein
Q8XT54	14917.01	7.66	Transmembrane protein
Q8XTB8	13892.54	9.23	Uncharacterized protein

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
Q8XTE1	32853.87	6.34	Uncharacterized protein
Q8XTF1	53831.36	8.48	Transmembrane protein
Q8XTH4	44798.81	10.67	nadh:flavin oxidoreductases oxidoreductase protein
Q8XTU8	35288.83	5.56	Utp-glucose-1-phosphate uridylyltransferase udp-glucose pyrophosphorylase protein
Q8XTX0	39553.19	5.12	Transmembrane protein
Q8XTY6	24072.8	10.24	Amino-acid transmembrane abc transporter protein
Q8XU31	71605.25	5.3	2',3'-cyclic-nucleotide 2'-phosphodiesterase transmembrane protein
Q8XUC7	28340.87	8.64	Periplasmic or secreted lipoprotein signal peptide
Q8XUM9	45134.96	7.78	Fad-dependent pyridine nucleotide-disulphide oxidoreductase; transmembrane protein
Q8XUT4	70222.96	6.05	Serine protease protein
Q8XUU4	39505.76	8.78	Outer membrane porin signal peptide protein
Q8XV98	39555.79	9.34	Porin signal peptide protein
Q8XVF2	26077.97	5.73	Ribulose-phosphate 3-epimerase protein
Q8XVH6	41294.31	8.46	Outer membrane porin signal peptide protein
Q8XVN8	15626.45	9.03	Outer membrane lipoprotein transmembrane
Q8XVV9	19831.77	9.5	Signal peptide protein
Q8XVZ3	38150.82	6.4	Twitching mobility protein transport fimbria
Q8XW22	53484.2	6.07	Protease signal peptide protein
Q8XW23	14704.01	10.38	Lipoprotein
Q8XWE8	21268.86	5.94	Superoxide dismutase
Q8XWH9	34463.41	6.7	Arylsulfatase (Aryl-sulfate sulphohydrolase) protein
Q8XWI2	51926.21	5.49	Hypothetical serine/threonine-specific phosphatase and bis(5-nucleosyl)-tetraphosphatase; transmembrane protein
Q8XWU0	21174.54	9.13	Bacterial outer membrane transmembrane protein
Q8XX33	15357.53	8.52	Signal peptide protein
Q8XX55	33636.69	9.22	Amino-acid-binding periplasmic (Pbp) abc transporter protein
Q8XX75	32679.47	4.68	Extracellular endo alpha-1,4 polygalactosaminidase or related polysaccharide hydrolase protein
Q8XYC3	28790.44	5.42	Uncharacterized protein
Q8XYC6	38050.66	7.77	Transcription regulator protein
Q8XYG2	37890.7	6.76	N-acetylmuramoyl-l-alanine amidase protein
Q8XYK2	74269.1	6.03	Exo-poly-galacturonosidase signal peptide protein
Q8XYK9	59571.22	6.26	Acyl-coa synthetase protein
Q8XYV4	32532.33	8.58	Phytoene synthase protein
Q8XZ01	20293.77	6.92	Polyhydroxybutyrate granule-associated protein (Phasin) phap1
Q8XZ53	172861.58	6.46	Transglycosylase protein
Q8XZK9	82127.61	8.03	Type III effector protein (Hlk1)
Q8XZX4	50586.13	5.99	Dihydrolipoyl dehydrogenase
Q8XZY7	52376.15	5.48	Glutamine synthetase
Q8Y008	29710.55	5.76	Myo-inositol catabolism protein
Q8Y079	20740.82	9.08	Peptidyl-prolyl cis-trans isomerase
Q8Y080	19721.17	6.23	Peptidyl-prolyl cis-trans isomerase

登录号 Access	分子量 Molecular weight	等电点 PI	蛋白质描述 Characteristics
Q8Y0Z1	20505.43	9.12	Signal peptide protein
Q8Y0Z3	23475.7	9.52	Outer membrane signal peptide protein
Q8Y119	14446.54	5.74	Transmembrane protein
Q8Y139	17661.91	4.57	Uncharacterized protein
Q8Y175	14772.69	9.78	Transmembrane protein
Q8Y191	37337.24	6.11	Purine nucleoside permease protein
Q8Y192	40581.9	8.82	Purine nucleoside permease protein
Q8Y1F4	18655.76	6.84	Peptidoglycan-associated lipoprotein
Q8Y1N4	30381.1	8.5	Pilus assembly cpab transmembrane protein
Q8Y1X9	17053.65	9.59	Type 4 fimbrial pilin signal peptide protein
Q8Y234	41281.35	5.61	Glycerophosphoryl diester phosphodiesterase, periplasmic protein
Q8Y255	34498.66	9.25	Amino-acid-binding periplasmic (Pbp) abc transporter protein
Q8Y2A7	86355.66	10.1	Mmpl exporter transmembrane protein
Q8Y2I4	59831.46	8.91	Peptidase transmembrane protein
Q8Y2I6	18404.1	4.91	Bacterioferritin
Q8Y2P9	25071.58	10.04	Cell division ftsn transmembrane protein
Q8Y2T5	156991.89	4.03	Calcium binding hemolysin protein
Q8Y2T6	120738.13	3.73	Calcium binding hemolysin protein
Q8Y377	101638.08	4.48	Hemolysin-type calcium-binding protein
Q8Y378	124446.62	4.03	Calcium binding hemolysin protein (Fragment)
Q8Y382	48901.78	7.79	Alkaline phosphatase signal peptide protein
Q8Y383	49600.5	5.55	Alkaline phosphatase lipoprotein transmembrane
Q9KGT9	28284.7	4.71	Flagellin
Q9RBW8	47861.79	8.41	TbuX